Reply to: Office Action of June 8, 2005

Title: METHOD FOR PURIFYING CANCER-SPECIFIC PROLIFERATING CELL NUCLEAR

**ANTIGEN** 

## **Listing of Claims**

Please amend the claims as follows:

- 1. (Amended) A method for purifying cancer-specific (csPCNA) comprising the steps of:
  - (A) obtaining a tissue or body fluid sample comprising csPCNA;
  - (B) contacting said sample with a peptide comprising the amino acid sequence
    - LeuLysGlnLeuAspAlaGlnGlnThrGlnLeuArgIleAspSerPhePheArgLeu AlaGlnGlnGluLysGluAspAlaLysArg (SEQ ID No:1),
    - wherein said peptide is immobilized on a solid support and binds to said csPCNA to form a peptide-csPCNA complex; and
  - (C) isolating csPCNA from said peptide-csPCNA complex so as to purify said csPCNA.
- 2. The method of Claim 1, wherein prior to step (B) the thus obtained tissue or body fluid sample of step (A) is subjected to a process comprising:
  - (1) homogenizing cells constituting said tissue or body fluid to obtain a homogenate (H);
  - (2) separating said H into a nuclear pellet fraction (NP) and a cytosolic fraction (S1);
  - (3) extracting nuclei from said NP to obtain a nuclear extract (NE);
  - (4) subjecting said S1 to centrifugation to obtain a postmitochondrial cytosolic supernatant (S2);
  - (5) subjecting said S2 to centrifugation to obtain a post-mitochondrial/post-microsomal cytosolic supernatant (S3);
  - (6) combining said NE and said S3 to form an NE/S3 fraction, applying the resulting NE/S3 fraction to a weak anion exchange matrix column and collecting the flow through (PCFT);
  - (7) applying the resulting PCFT to a hydrophobic

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chromatography matrix column, eluting the column with buffer comprising ethylene glycol and collecting the eluant (PSE);

- (8) dialyzing out ethylene glycol present in the PSE to obtain a dialyzate; and
- (9) applying the resulting dialyzate to a strong anion exchange matrix column, eluting with a dialyzate buffer comprising a salt gradient, and collecting and pooling PCNA-containing fractions to obtain said sample.
- 3. (Amended) The method of Claim 1 or 2, wherein said tissue or body fluid sample of step (A) further comprises native PCNA (nPCNA), said nPCNA does not bind to said peptide in step (B), whereas said csPCNA binds to said peptide in step (B) to form a peptide-csPCNA complex and in step (C) isolating csPCNA is effected using an elution buffer whereby csPCNA is eluted from said csPCNA-complex.
- 4. An immunoassay for detecting csPCNA comprising:
  - (1) contacting a test sample with a peptide comprising the amino acid sequence

LeuLysGlnLeuAspAlaGlnGlnThrGlnLeuArgIleAspSerPheP heArgLeuAlaGlnGlnGluLysGluAspAlaLysArg (SEQ ID No:1), which has been immobilized on a solid support so as to bind csPCNA to said peptide to form a peptide-csPCNA complex; and

- (2) contacting said peptide-csPCNA complex with an anti-PCNA antibody and detecting binding of said antibody to said complex.
- 5. The immunoassay of Claim 4, wherein said assay is an ELISA and said antibody is labeled with a detectable enzyme.

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6. (Amended) The immunoassay of Claim 5, wherein said enzyme is <u>at</u>

<u>least one of horse radish peroxidase and alkaline phosphotase.</u>

- 7. (Amended) The immunoassay of Claim 5 4, wherein said peptide is a fusion protein comprising said peptide and at least one of Glutathione-S-Transferase, Calmodulin Binding protein, and oligo (6X) histidine.
- 8. (Amended) The immunoassay of Claim 7, wherein said fusion protein is biotinylated and immunobilization immobilized on said solid support via streptavidin-coated on said solid support.
- 9. (New) The method of Claim, 1, wherein the tissue or body fluid sample obtained is afflicted with a cancer selected from the group consisting of carcinomas, sarcomas, lymphomas, or leukemias.
- 10. (New) The method of Claim 9, wherein the tissue or body fluid sample obtained is selected from the group consisting of cervical, mammary glands, esophageal, glial cells, lung, stomach, intestine, prostate, white blood cells, urine, serum, or whole blood.
- 11. (New) The method of Claim 1, wherein said peptide is a fusion protein comprising said peptide and at least one of Glutathione-S-Transferase, Calmodulin Binding protein, and oligo (6X) histidine.
- 12. (New) The method of Claim 1, wherein said isolated and purified csPCNA is used to produce antibodies (monoclonal or polyclonal) specific for csPCNA.
- 13. (New) The method of Claim 3, further comprising the step of identifying the site(s) on the PCNA polypeptide that is (are) modified in nPCNA and lacking from csPCNA.

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14. (New) The method of Claim 13, further comprising the step of identifying the metabolic pathways that mediate the addition or removal of this (these) post-translation modifications.

- 15. (New) The method of claim 1, further comprising the step of developing specific inhibitors for csPCNA.
- 16. (New) The immunoassay of Claim 4, wherein the test sample is afflicted with a cancer selected from the group consisting of carcinomas, sarcomas, lymphomas, or leukemias.
- 17. (New) The immunoassay of Claim, wherein the test sample is obtained by selecting from the group consisting of cervical, mammary glands, esophageal, glial cells, lung, stomach, intestine, prostate, white blood cells, urine, serum, or whole blood.
- 18. (New) The immunoassay of Claim 4, wherein said immunoassay is selected from the group consisting of radio-immuno assay, dot blot assay, slot blot assay, immunoprecipitation, protein quantification, imunno-PCR, or Western blot.